**Facile synthesis, spectroscopic and single crystal X-ray characterization of [Co(bpy)2CO3](C7H4NO3S).3H2O complex with antibacterial and anticancer screening**

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**Abstract**

The facile synthesis of cobalt (III) complex [Co(bpy)2CO3](C7H4NO3S).3H2O (where bpy= 2,2’-bipyridine, C7H4NO3S= Saccharinate) has been performed by mixing solutions of [Co(bpy)2CO3]Cl.3H2O and Sodium saccharinate in equimolar ratio in water. The composition of structure was strengthened by elemental and spectroscopic studies such as CHN, FT-IR, 1H NMR and UV-Vis. Single crystal X-ray structure investigation revealed one [Co(bpy)2CO3]+ cationic counterpart, one saccharinate anion in outer sphere and three water molecules of crystallization. Robust hydrogen bonding interactions were witnessed from structural data. The presence of saccharinate ion in the complex was stemmed by presence of CO and SO2 stretching vibration. Further, the coordination structure was supported by the TGA studies. The antibacterial screening of complex was examined against the specific Gram-positive and Gram-negative bacteria by disc-diffusion method. MIC was determined by utilizing broth-dilution method. Broad spectrum activity was witnessed for the complex as it was active against both bacterial strains. Furthermore, the complex showed growth-inhibitory effect against PANC-1 cells.

**Keywords: -** CoCl2.6H2O, Saccharinate, Anion-receptor, X-ray crystallography, Antibacterial, Anticancer

**1. Introduction**

The exploration into anion binding studies in past years has encouraged the scientists to the fabrication of supramolecular architectures with unique packing patterns and hydrogen bonding interactions. The synthetic methodology utilized is purposely incorporating hydrogen bonding groups into ordered receptor molecules. Therefore, innovative receptors with the precise functional moieties need to be designed to systematically bind with biologically, industrially and environmentally important anions [1,2]. These anions possess diverse shapes and geometries in contrast to cations which challenge their binding to receptors [3].Thus fabrication of supramolecular architectures requires the carefully chosen precise supramolecular synthon (host) which forms hydrogen bonding interactions with the anions (guest) [4,5].

In the literature, several anion receptors have been fabricated [6,7] but our selection of [Co(bpy)2CO3]+ as anion receptor stemmed from its potency to form robust hydrogen bonding interactions from C=O (carbonate group). In continuance to the preceding work [8,9] in this paper, the binding of [Co(bpy)2CO3]+ receptor with industrially important saccharinate anion through hydrogen bonding interactions is studied by X-ray structural study. This anion is selected because it proposes probable coordination centres such as imino nitrogen, two sulfonyl and one carbonyl oxygen atom for binding with the selected receptor. So, it possess diverse and remarkable coordination chemistry as it causes robust hydrogen bonding interactions in solid-state architectures. It is well acknowledged as artificial sweetener and polyfunctional ligand [10,11]. Thus [Co(bpy)2CO3]+ metal complex was fabricated as receptor for Saccharinate anion i.e. preparation, spectroscopic and thermal studies of designed complex with composition [Co(bpy)2CO3](C7H4NO3S).3H2O (where bpy = 2,2’-bipyridine, C7H4NO3S= Saccharinate).

**2. Experimental**

**2.1 Materials**

Chemicals like Sodium Saccharinate and 2,2’-bipyridine (bpy) were acquired from Merck Chemical Co. and CoCl2.6H2O was acquired from Sigma Aldrich, UK. [Co(bpy)2CO3]Cl.3H2O was synthesized with somewhat altering the synthesis specified in literature [12]. This complex was further engaged for constructing the title complex at room temperature.

**2.2 Synthesis**

**2.2.1 Synthesis of [Co(bpy)2CO3](C7H4NO3S).3H2O**

Aqueous solution of 0.520 gram of [Co(bpy)2CO3]Cl.3H2O was mixed with an equivalent ratio of aqueous solution of 0.242 gram of sodium saccharinate and set aside at room temperature for evaporation. After 5 days, maroon coloured crystals of X-ray diffraction quality were visualized. The crystals attained by filtration were left for drying in the air.

**2.3 Instrumentation**

Perkin-Elmer 2400 CHN analyzer, EI 2375 double beam spectrophotometer, Bruker Advance NMR Spectrometer, Perkin-Elmer Spectrum RX FT-IR system, TGA detector model SDT Q600 instrument.

**2.3.1 X-ray Crystallography**

Crystallographic study of the designed complex was evaluated on a Super-Nova X-ray diffractometer which was equipped with a HyPix3000 (CCD plate) detector. The data are measured by retaining the crystal on Hampton cryoloops with a monochromated Mo-Kα (λ = 0.71073 Å) X-ray source. Olex2 [13] was employed for data collection and space group determination. The program SIR2004 [14] and ShelXL [15] were employed for solving the structure.

**2.3.2 Antibacterial assay**

**2.3.2.1 Disc-diffusion method**

The *in vitro* antibacterial analysis against *Bacillus subtilis* and *Escherichia coli* was elucidated by disc diffusion method [16]. Nutrient agar media was employed for bacteria culture for 12 hours at temperature 37°C. The title complex was dissolved in DMSO (1mg/ml) and impregnated to the filter paper (Whatsmann no. 1) sterile disc of 5mm diameter and dispensed to the nutrient agar plate. The diameter of inhibition zone in mm around the impregnated disks was measured relative to the positive control after 12 hours. The minimum inhibitory concentration (MIC) of designed complex was determined by utilizing micro broth dilution method.

**2.3.3 Cytotoxic activity**

**Cell culture and growth medium**

The effect of complex on the viability of PANC-1 was determined using the colorimetric MTT assay. The cells were cultured in DMEM medium (Dulbecco’s Modified Eagles medium) containing 10% FBS, 100 µg/mL streptomycin, 0.06 mg/mL penicillin and 0.25 µg/mL amphotericin B.

MTT cytotoxicity assay: Cells were seeded in 96 well tissue culture treated plates (1×104 cells/well). The cells were incubated with respective concentrations (325, 650, 468 µg/mL) of the complex for 24 and 48 hours at 37 °C and 5% CO2 supply in a CO2 incubator. Upon completion of incubation, 20 µl of MTT solution (5mg/mL) was added to each well. After 4 hours, the medium was removed and 200µl of DMSO was added to solubilize formazan crystals. The intensity was measured at 570 nm by Multiskan FC Microplate photometer (Thermo Fisher Scientific). The percentage growth inhibition was calculated from the dose-dependent curves for the cell line.

**3. Results and discussion**

**3.1 Spectral characterization**

The FT-IR absorption spectrum has been acquired in the range 4000-400 cm-1 and the assigned distinctive bands for the complex are depicted in S1. The broad peak at 3416 cm-1 ascribes to ν(OH) vibrations from water molecules [17]. Saccharinate anion was remarkably characterized by strong IR peaks due to the existence of carbonyl and sulfonyl group. The *ν*(CO) stretching vibration was elucidated at 1677 cm-1 which was relatively comparable to the earlier vibrational analysis for various metal saccharinates [18,19]. The vibration for *ν*(CO) mode was predicted at 1725 cm-1 for free saccharin [20] which was quite lowered. This decrease in wavenumber may be attributed to metal–saccharinate bonding (through nitrogen). The sulphonyl stretching, *ν*(SO2) mode for the asymmetric and symmetric vibrations was visualized as distinctive bands around 1265 cm-1 and 1144 cm-1 and the values were quite analogous to the free saccharin absorption band (1360 and 1180 cm−1) [20].

1H NMR spectra was visualized in DMSO-*d6* as a solvent and internal reference TMS. From 1H NMR, two doublets and two triplets peaks were visualized suggestive of two bipyridine rings. The aromatic protons were witnessed in the range 8.96 -8.13ppm [21]. The 1H NMR demonstrated the probable signals for the saccharinate ligand with multiplets between 7.66-7.57 ppm (S5). Related peak for saccharinate was reported in the literature [22]. The newly designed complex saccharinate was witnessed to have total of 20 protons and starting material was witnessed to have a total of 16 protons. 1H NMR was relatively consistent with the suggested stoichiometry of saccharinate complex.

The UV-Vis spectroscopic analysis was visualized in the 200-800 nm interval in water. The first absorption band was notified at 303 nm for bipyridine ligand (π-π\*) transitions. Corresponding absorption peak for bipyridine was visualized in literature [23]. Another absorption band at 401 nm for (Co (III) complex) was visualized.

**3.2 Thermal analysis**

The study of thermal breakdown of this complex was accomplished in temperature range 25-1000 °C under nitrogen atmosphere (S4). The results are comparable with the theoretical formula proposed by elemental analysis. The complex is constant upto 160°C and its decomposition initiated at this point. The first mass loss was 8.79% equivalent to the three water moleculesloss (*ca.* 8.78%). At a temperature 310°C, the decomposition of saccharinate molecule occurs with experimental mass loss (30.65%) which is relatively nearer to the calculated one 30.68%. The decomposition temperature of saccharinato ion in title complex ensues at 23°C higher temperature in contrast to pure saccharin (287°C) which might be conveyed to the robust interaction between saccharinate and Co(III) ion [24]. Further heating caused the complete decomposition.

**3.3 Crystal structure description**

X-ray crystallography technique was employed for visualizing the structural features of this novel architecture. The complex crystallizes in triclinic *P-1* space group. The asymmetric unit comprised of [Co(bipy)2CO3]+ in inner sphere and saccharinate anion and three guest water molecules in outer sphere (Figure 1). The Co(III) ion has distorted octahedral geometry comprising of two bidentate 2,2'-bipyridine and one bidentate symmetrical chelating carbonate anion. The bipyridine binds through N1, N2 , N3 and N4 besides O1 and O2 carbonate oxygens and the carbonate binds through Co–O1 1.8872(15) and Co–O2 1.8872(14) Å. The bond lengths of C1–O1 of 1.329 (3) Å and C1–O2 of 1.317(3) Å are approximately identical assisting in chelation with aid of carbonate group. The Co (III) metal centre possesses N1, N2, N4, O1 at the equatorial positions and N3, O2 at the axial position. The extreme deviation from the consistent octahedral geometry in *cis* angle (N1−Co1−O2) is 9.54° and in *trans* angle (N1−Co1−O1) is 13.07° from the ideal value of 90° and 180° correspondingly (Table 2). Both the chelate rings are planar, rigid and nearly perpendicular to each other having a dihedral angle of 92.81**˚**.

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**Figure 1.**ORTEP diagram of the complex salt with partial atom numbering scheme. The displacement ellipsoids are drawn at 40% probability (guest water molecules are removed for clarity).

Crystallographic data and structure refinement are précised in Table 1-2. In the complex, the bond lengths and bond angles of Co ̶ Nbipyridine, Co ̶ Ocarbonato are comparable to those of similar complexes in literature [25].

In the complex, the saccharinate ion constitutes a molecular plane which dissects the atoms in plane and reflects two oxygen atoms. The hydrogen bonding associations are illustrated in Figure 2. Non-covalent interactions such as C‒H···O (bpy/saccharinate) stabilize the crystal lattice.

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**Figure 2.**ORTEP diagram of complex salt depicting chains of hydrogen bonding between Co complex and saccharinate ion

When the complex is observed down *a* axis for packing (Figure 3), a bilayered structure was envisaged where set of cations creates every layer in 180° manner with the anionic moieties off-sandwiched among two cation counterparts facing to different directions viewing along *b*-axis. π–π stacking of 3.862 Å is visualized for bipyridine. The anionic counterpart has small contacts as 3.433 Å and 3.969 Å to both the bipyridine components. The three guest water molecules formed robust hydrogen bond interactions such as O‒H···O (water/saccharinate), O‒H···O (water/water) and O‒H···O (water/carbonate) thus stabilising the lattice.

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**Figure 3.** The lattice along *a*-axis depicting a chain of layers of a cationic complex salt

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**Figure 4.** Ball and stick model of the title complex

**Table 1:** Crystal detailand refinement particular of the title complex.

|  |  |
| --- | --- |
| Empirical formula | C28H20CoN5O6S |
| CCDC | 2014541 |
| *M* (g mol-1) | 614.49 |
| *T* (K) | 293(2) |
| λ (Å) | 0.71073 |
| Crystal system | Triclinic |
| Space group | P-1 |
| *a*/Å | 7.0966(2) |
| *b*/Å | 14.1006(4) |
| *c*/Å | 14.2948(4) |
| *α*/° | 80.697(2) |
| *β*/° | 83.560(2) |
| γ/° | 82.890(2) |
| *V*/Å3 | 1394.54(7) |
| *Z* | 2 |
| *ρ*calc/cm3 | 1.463 |
| *μ*/mm‑1 | 0.741 |
| F(000) | 630.0 |
| Crystal size/mm3 | 0.21 × 0.18 × 0.11 |
| Unique reflections | 5949 |
| *R*(int) | 0.0601 |
| GOF on *F*2 | 1.109 |
| *R*1 [*I*> 2*σ*(*I*)] | 0.0410 |
| *wR*2 [*I*> 2*σ*(*I*)] | 0.1069 |

**Table 2**: Selective bond lengths and bond angles around central Co (III) ion in title complex

|  |  |  |  |
| --- | --- | --- | --- |
| **Bond distances (Å)** | | | |
| Co1 O1  Co1 O2  Co1 N1  O2 C1 | 1.8872(15)  1.8872(14)  1.9385(18)  1.317(3) | Co1 N2  Co1 N3  Co1 N4  O1 C1 | 1.9187(18)  1.9409(17)  1.9302(17)  1.329(3) |
| **Bond angles (˚)** | | | |
| N1 Co1 N3  N1 Co1 C1  N2 Co1 C1  N2 Co1 N1  N2 Co1 N3  N2 Co1 N4  N4 Co1 N3  N4 Co1 N1  N4 Co1 C1  O1 Co1 N1  O1 Co1 N2 | 92.79(7)  134.09(8)  92.81(8)  82.73(8)  96.41(7)  178.98(7)  82.85(7)  97.99(7)  87.20(7)  168.14(7)  92.53(7) | N3 Co1 C1  O2 Co1 C1  O2 Co1 N4  O2 Co1 N3  O2 Co1 N2  O2 Co1 N1  O1 Co1 O2  O1 Co1 C1  O1 Co1 N4  O1 Co1 N3 | 133.04(8)  34.57(7)  91.14(7)  166.94(7)  89.45(7)  99.54(7)  69.44(6)  34.93(7)  86.90(7)  98.56(7) |

**3.4 Antibacterial activity**

**3.4.1 Agar well diffusion method**

The inhibition zone values assessed from the triplicate experiments depicted the inhibition diameter of 52 mm for standard drug Ampicillin. From the results, the complex was visualized with modest activity against the selected gram positive and gram negative bacteria. The inhibition zone diameter value for complex was found out to be 15 mm and 12 mm in contrast to bipyridine ligand [44] which were observed to have inhibition zone values of 14 mm and 10 mm for *E. coli* and *B. Subtillis* respectively. The better activity of complex than ligand bipyridine might be due to chelation of metal ion with the ligand [27].

**Minimal Inhibitory Concentration (MIC)**

The MIC data against bacterial culture of *B. subtillus* and *E. coli* is summarized in Table 3 respectively. MIC50 of complex against *B. Subtillus* was 0.0521 mg/mL and for *E. coli* the MIC50 was 0.0471 mg/mL.

**Table 3**: Antibacterial activity of complex against *Bacillus subtillus and Escherichia coli* utilizing microbroth dilution assay (MDA)

|  |  |  |  |
| --- | --- | --- | --- |
| **Complex** | ***Bacillus subtillus*** | ***Escherichia coli*** | **Ampicillin** |
| **MIC50 (mg/mL)** | 0.0521 | 0.0471 | 0.00004 |
| **MIC90 (mg/mL)** | 0.189 | 0.178 | 0.0001 |
| **MIC range (mg/mL)** | 0.0098-0.190 | 0.009-0.178 | 0.00002-0.0001 |

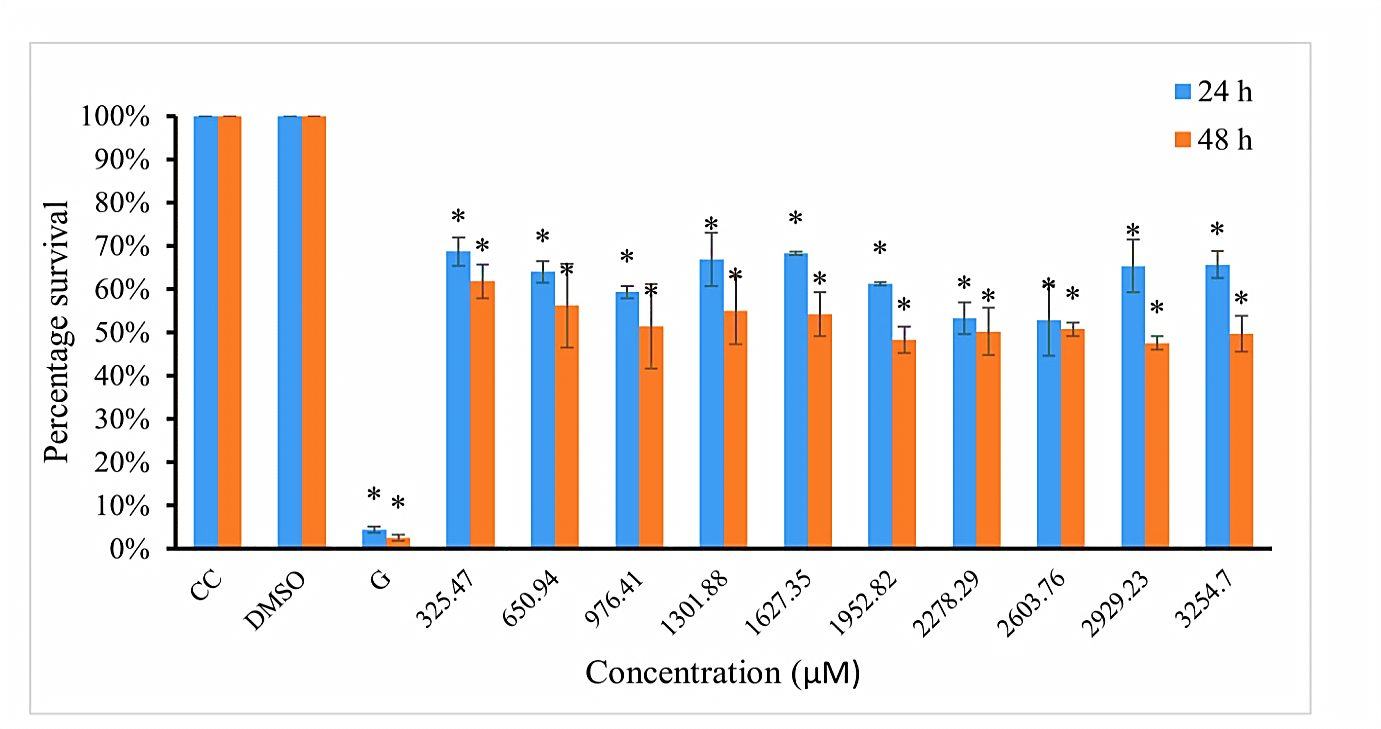
**Minimal Bactericidal Concentration (MBC)**

For MBC measurement, the test dilution was subcultured on to a fresh solid medium which is drug-free and incubating it further for 24 h. MBC is thus defined as the highest dilution that generated no single bacteria colony onto a solid medium. The MBC of the complex for *B. subtillus* bacteria was 0.99 mg/mL and for *E. coli* was 1.2 mg/mL respectively.

**3.5. Evaluation of cytotoxicity**

The effect of complex on the viability of PANC-1 cells was determined by a rapid and quantitative colorimetric MTT assay. This colorimetric method helps in detecting the living cells. The cells were treated with varying concentrations of title complex in the range 325.4 to 3254.7 µM

In the reduction in cell survival percent, it was revealed from the graph in Figure 3 the complex was found to be most active at a concentration of 2278.29 μM, as the percent cell viability reaches 53% and this indicated that the complex has caused the death of most of the PANC-1 cells at this concentration after 24 hour. The IC50 value at 48 hour (976.41μM) was observed to be lower than 24 hour (2278.29μM) indicating the time dependence of cytotoxicity.



**Figure 3. Comparative percentage survival of the PANC-1 cells at 24 h and 48 h with varying concentrations of title complex. The results were expressed as Mean±SD of % cell survival from the triplicate experiments (\* represents substantial differences between the control and experimental group i.e. P < 0.05)**

**4. Conclusion**

This paper highlights the design, synthesis and structure interpretation of cobalt (III) complex [Co(bpy)2CO3](C7H4NO3S).3H2O. This complex was spectroscopically and structurally characterized. Octahedral geometry was allocated to Co(III) ion in the complex by the single crystal X-ray analysis. The *in vitro* cytotoxicity of complex was performed by cell viability MTT assay on PANC-1 cell lines and it indicated the time dependence of cytotoxicity. *In vitro* antibacterial study of complex indicated that the complex have higher activity than respective ligand bipyridine against all bacteria. Antibacterial analysis depicted that the complex has broad spectrum activity as it is susceptible against both gram negative and gram positive bacteria. The acquired biological results recommended that the novel architecture can counter antibiotic resistant bacteria.

**5. Supplementary data**

For analysing the Crystallographic data for the crystal structure, the Deposition number CCDC 2014541 can be quoted for obtaining the data from the Cambridge Crystallographic Data Centre.

**6. Acknowledgement**

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